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Effects of experimental chlorate preparations as feed and water supplements on *Escherichia coli* colonization and contamination of beef cattle and carcasses

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Abstract

The effects of feed or water administration of experimental chlorate preparations on recovery of generic *Escherichia coli* and *E. coli* O157:H7 from the gut, hide and carcasses of feedlot cattle were tested. Sixty-four naturally colonized cattle were randomly assigned to one of four feed treatments with or without a 12 h chlorate-containing water treatment. An analysis of variance revealed a main effect of feed treatment ($P = 0.002$) on generic *E. coli* concentrations in feces collected before shipment to slaughter. Main effect means were 5.57, 4.75, 5.00 and 4.08 $\log_{10}$ cfu/g for animals fed an experimental chlorate product at 0, 0.01% in last meal, 0.01% for last 5 d and 0.05% of body weight in last meal, respectively. A main effect of feed treatment was observed ($P = 0.041$) on generic *E. coli* concentrations in feces collected at slaughter (means for the respective treatments were 4.92, 3.57, 3.98 and 3.20 $\log_{10}$ cfu/g) as well as on numbers of generic *E. coli* recovered ($P = 0.034$) from hide swabs collected at the rump (means for the respective treatments were 4.86, 3.92, 3.87 and 4.06 $\log_{10}$ cfu/swab). A main effect of water treatment was observed ($P < 0.016$) on generic *E. coli* concentrations in rumen contents (3.44 vs. 2.72 cfu $\log_{10}$ cfu/g for animals administered 0 or 2500 ppm active chlorate ion, respectively). Logistic regression analysis revealed a main effect of feed treatment ($P < 0.001$) on the incidence of *E. coli* O157:H7 recovered from feces collected at slaughter (75%, 33%, 20% and 25% for animals fed an experimental chlorate product at 0%, 0.01% in last meal, 0.01% for last 5 d and 0.05% of body weight in last meal, respectively). Animals exhibited no symptoms of chlorate toxicity and negative effects on feed or water intake or animal performance were not observed.

Keywords: Beef cattle; *Escherichia coli*; Experimental chlorate product; Feed additive; Water additive

1. Introduction

Enterohemorrhagic *Escherichia coli* strains such as *E. coli* O157:H7 are important foodborne pathogens. In the United States, an estimated 73,000 human infections caused by *E. coli* O157:H7 occur each year (Mead et al., 1999). Cattle are an important reservoir of *E. coli*

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O157:H7 (Orskov et al., 1987; Rasmussen et al., 1993), with practically all herds containing at least some colonized animals and, depending on the season, with incidences within herds $\geqslant$30% (Elder et al., 2000). Despite implementation of numerous post harvest interventions by beef processors (Bacon et al., 2000; Cutter and Rivera-Betanourt, 2000), costly product recalls (FSIS, 2002, 2003a, b; Thomsen and McKenzie, 2001) and disease outbreaks (Vugia et al., 2003) resulting from consumption of food contaminated with this pathogen continue to occur. Not surprisingly, there is considerable interest in the development of pathogen reduction strategies that can be applied to the live animal before slaughter, particularly since some risk assessments predict that such interventions would significantly reduce human exposures to this pathogen (Hynes and Wachsmuth, 2000).

Recently, we reported that oral administration of low levels of an experimental chlorate product selectively reduced concentrations of *E. coli* and *Salmonella* in the gut of pigs, cattle and sheep (Anderson et al., 2001a, b, 2002; Callaway et al., 2001, 2002, 2003; Edrington et al., 2003). Mechanistically, the chlorate is catalytically reduced to cytotoxic chlorite by the bacterium's respiratory nitrate reductase enzyme (Anderson et al., 2000; Stewart, 1988). A particularly attractive feature of this intervention is that it not only reduces gut concentrations of *E. coli* and *Salmonella* in the gut but it is harmless to most commensal and mutualistic anaerobes (competitive exclusion bacteria) that lack respiratory nitrate reductase (Anderson et al., 2000, 2001b). Whereas results from these earlier studies proved the concept of this technology (Anderson et al., 2002; Callaway et al., 2002), the present study was conducted to test the practicality and efficacy of administering experimental chlorate preparations to beef cattle as feed and water supplements under conditions more likely to be encountered during the finishing of beef cattle.

2. Materials and methods

2.1. Animals and experimental design

Sixty-four head of feedlot heifers of Mexican origin and averaging 402 ± 5.1 (SD) kg were sorted at random and without regard to *E. coli* O157:H7 culture status to one of eight treatments in a two $\times$ four factorial design (two drinking water treatments and four feed treatments; two heifers per treatment) that was replicated four times thus bringing the final number of animals to eight per treatment. Previous studies have demonstrated that an $n \leqslant 8$ animals per treatment was more than sufficient to detect differences in naturally colonized and experimentally infected animals (Anderson et al., 2001b, 2002; Callaway et al., 2002, 2003; Edrington et al., 2003). Each replicated trial was conducted at 2 week intervals. The two water treatment factors were a control treatment containing no chlorate (0X) or a specially prepared 1X solution containing 2500 ppm active ion which were provided to the animals during the last 12 h prior to being shipped to slaughter. The factors for feed treatment were the provision of an experimental chlorate product (EKA Chemicals, Inc., Marietta, GA) at 0, 0.01% of body weight for the last day on feed, 0.01% of body weight for the last 5 days on feed or 0.05% of body weight for the last day on feed. The experimental chlorate product, containing less than 40% active ion and 4% nitrate ion by weight (the latter to induce expression of respiratory nitrate reductase), had been prepared to possess rumen bypass characteristics to increase delivery of the active ion to the lower gut (Edrington et al., 2003).

Beginning 1 week prior to administration of the different treatments, each animal was weighed and moved to a separate pen where they were adapted to their pen and finishing diet (Table 1). Meals (equal to 1.25% of body weight on an as fed basis) were fed twice daily, at 08:00 and 16:00, except on the day before slaughter, at which time feed was offered during the morning feeding only. Feed intake was measured during the 5 days immediately preslaughter by recovering and weighing feed refusals immediately before the next meals offering, or in the case of the last meal, after the animals were shipped to slaughter. Homogenous portions of feed offered and recovered were dried at 100 °C until achieving a constant weight to determine dry matter percentages. Each pen was fitted with a tank-type watering trough capable of holding approximately 115 l so that each animal could individually be provided ad libitum access to drinking water or treatments and volume consumed during the 12 h water treatment period was measured by use of a precalibrated measuring gauge.

Four hour after offering of the last meal, the cattle were weighed and then withheld from water until 16:00 h

Table 1
Ingedient composition of feedlot diet provided to cattle

Ingredient (As fed basis)	%
Steam rolled corn	79.50
Cottonseed meal	7.39
Vitamin premix[a]	0.05
Trace mineral premix[a]	0.05
Urea	0.98
Cottonseed hull	6.49
Soy oil	3.61
Limestone	1.43
Salt	0.50

[a]Vitamin Premix 6905; Trace Mineral Premix 6962 (Producers Cooperative Association, Bryan, Texas, USA).

at which time they were again allowed access to water that containing the respective treatments (0 or 1X). Twenty hours after receiving their last meal, the cattle were shipped approximately 150 km to a slaughter facility where they were processed 4–6 h later. Fresh feces collected from each animal immediately prior to shipping, hide and carcass swabs, rumen contents and feces collected from offal at slaughter, but prior to any post harvest interventions, were cultured for wildtype *E. coli* and *E. coli* O157:H7 as described below. Swabs were collected using Speci-Sponges (Nasco, Fort Atkinson, Wisconsin, USA) hydrated with 20 ml Butterfield's buffer. Swabs of the hide were collected from 100 cm^2 areas located between the front shoulder blades along the back midline, and on the left rump just below the pelvic bone. Carcass swabs were collected from a 100 cm^2 area located between the bung and hock. Fresh feces collected from cattle at the beginning of each replicate (prior to administration of treatments) were also cultured for *E. coli* O157:H7.

2.2. Bacteriology and analytical

Gut samples and swabs were cultured quantitatively for generic *E. coli* via direct plating of serial dilutions to Sorbitol MacConkey and M-endo agars (Bectin Dickinson, Sparks, Maryland, USA), respectively. All incubations were performed at 37 °C for 18–24 h. All gut and swab samples were also cultured qualitatively for *E. coli* O157:H7 using the immunomagnetic separation method of Elder et al. (2000). Concentrations of volatile fatty acids in gut contents collected at slaughter were determined by gas chromotography (Hinton et al., 1990).

2.3. Statistical analysis

Intake, weight gain and concentrations of volatile fatty acids and log transformations of generic *E. coli* counts were analysed for treatment effects by analysis of variance (STATISTIX® 8, Analytical Software, Tallahassee, Florida, USA). Data were analysed with water treatments, feed treatments, replicate and their interactions in the model. Means were further separated using an LSD All-Pairwise Comparisons Test. Incidence of *E. coli* O157:H7 was tested for potential treatment effects by logistic regression with probability estimates obtained by deviance tests using STATISTIX® 8.

3. Results

A main effect of feed treatment ($P = 0.002$) on generic *E. coli* concentrations in feces collected before shipment was observed. Main effect means were 5.57, 4.75, 5.00 and 4.08 $\log_{10}$ cfu/g for animals fed an experimental chlorate product at 0, 0.01% in last meal, 0.01% for last 5 days and 0.05% of body weight in last meal, respectively (SEM = 0.33). Main effects of feed treatment were also observed ($P = 0.041$) on *E. coli* concentrations in feces collected at slaughter. Main effect means for the respective treatments for feces collected at slaughter were 4.92, 3.57, 3.98 and 3.20 $\log_{10}$ cfu/g (SEM = 0.63). Main effects of water treatment, replicate or the possible interactions were not observed ($P > 0.05$) on fecal *E. coli* concentrations. A main effect of water treatment ($P = 0.016$) but not feed treatment, replicate or the possible interactions ($P > 0.05$) was observed on *E. coli* concentrations in rumen contents (main effect means for water treatment were 3.44 vs. 2.72 $\log_{10}$ cfu/g for animals administered 0 or 2500 ppm active chlorate ion, respectively; SEM = 0.42). A main effect of feed treatment ($P = 0.034$) but not water treatment or any possible interaction ($P > 0.05$) on numbers of *E. coli* recovered from hide swabs collected at the left rump was observed. Main effect means of feed treatment for the rump hide swabs were 4.86, 3.92, 3.87 and 4.06 $\log_{10}$ cfu/swab for animals fed an experimental chlorate product at 0, 0.01% in last meal, 0.01% for last 5 days and 0.05% of body weight in last meal, respectively (SEM = 0.23). Replicate effects ($P < 0.001$) on recovery of *E. coli* from swabs from the hide swabs of the back midline and left rump areas and carcass swabs were observed, with higher recoveries obtained during the second and third replicates than from the first and fourth replicates (data not shown). While water treatment tended ($P = 0.08$) to reduce numbers of *E. coli* recovered from carcass swabs no other main effects of feed treatment, water treatment or any possible interaction were observed ($P > 0.05$) on numbers of *E. coli* recovered from hide swabs of the back midline or from carcass swabs. Further separation of means using an LSD All-Pairwise Comparison showed some additional effects of feed and water treatments on recovery of generic *E. coli* from gut contents and swabs and these are presented in Figs. 1 and 2. The data presented here were obtained from enumeration of colonies on M-endo agar, however, analysis of counts obtained from Sorbitol MacConkey agar yielded similar results except a trend ($P = 0.07$) for an effect of feed treatment existed for reduced recovery of *E. coli* from the carcass swabs (data not shown).

Qualitative recovery of *E. coli* O157:H7 was variable, with 35 of the 64 (55%) animals yielding a culture positive fecal specimen before initiation of treatment, and logistic regression analysis revealed no bias in treatment allocation ($P > 0.05$). Logistic regression analysis revealed a water treatment X feed treatment interaction on recovery of *E. coli* O157:H7 from feces collected post treatment but before shipment to the abattoir ($P = 0.002$) but no effects on recoveries from rumen contents or hide and carcass swabs collected at slaughter (Figs. 3 and 4). An effect of feed treatment

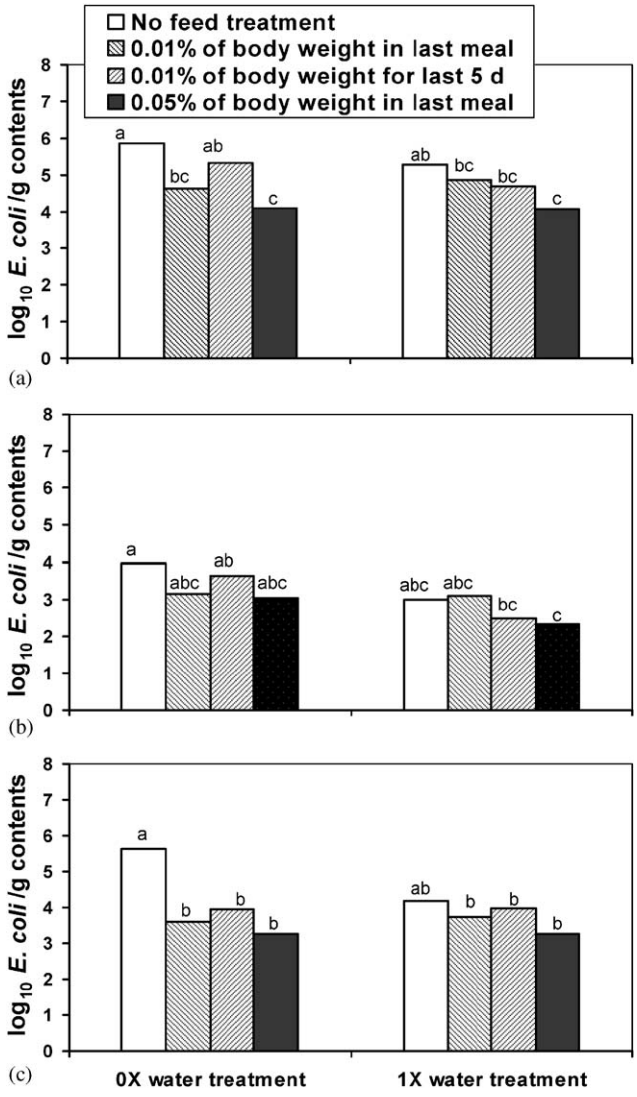

Fig. 1. Effects of administering sodium chlorate preparations in drinking water and/or feed on generic *Escherichia coli* concentrations in feces collected post treatment but immediately before shipment to slaughter (a) and in rumen contents (b) and feces collected at slaughter (c). Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1X water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests were done by analysis of variance and data presented are LSD All-Pairwise Comparisons of means. Columns with unlike superscripts differ at $P<0.05$. SEM = 0.33, 0.42 and 0.63 for data in graphs A, B and C, respectively.

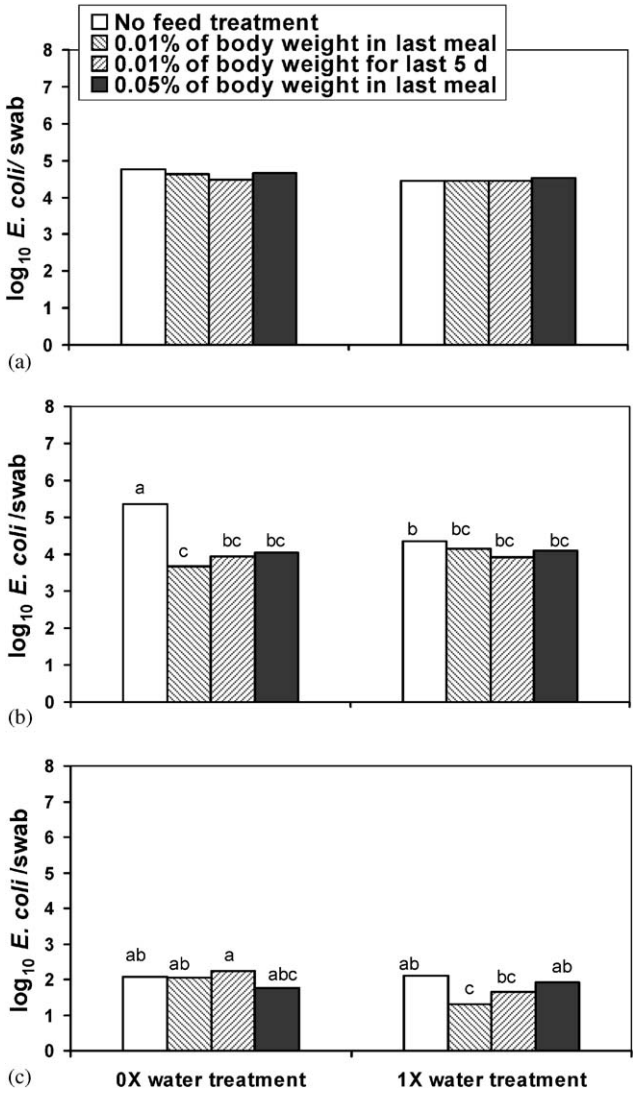

Fig. 2. Effects of administering sodium chlorate preparations in drinking water and/or feed on generic *Escherichia coli* concentrations in swabs collected from the back midline (a) and left rump area (b) of the hide and from the bung/hock area of the carcass (c) collected at slaughter. Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1 X water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests were done by analysis of variance and data presented are LSD All-Pairwise Comparisons of means. Columns with unlike superscripts differ at $P<0.05$. SEM = 0.19, 0.23 and 0.20 for data in graphs A, B and C, respectively.

($P<0.001$) was observed on recovery of *E. coli* O157:H7 from feces collected at the slaughter plant, with treated cattle being less likely to yield a culture positive specimen (Fig. 3).

Feed intake was lowest, but not significantly, among cattle consuming no chlorate and although no effect ($P>0.05$) of feed or water treatment was observed, feed intake was lower than anticipated (Table 2). As a consequence, the cattle lost 7.0 ± 1.5 kg live BW (mean $\pm$ SE) during their 2 weeks at our facilities but an analysis of variance revealed that this was not effected ($P>0.05$) by either feed or water treatment. No adverse effects due to feed or water treatment were observed on water consumption during the 12 h period immediately prior to transport to the slaughter facility (Table 2). Main effects of replicate or any possible interaction on feed or water intake were not observed ($P>0.05$). A main effect of water treatment was observed on rumen concentrations of acetate ($P=0.006$), propionate ($P=0.005$) and butyrate

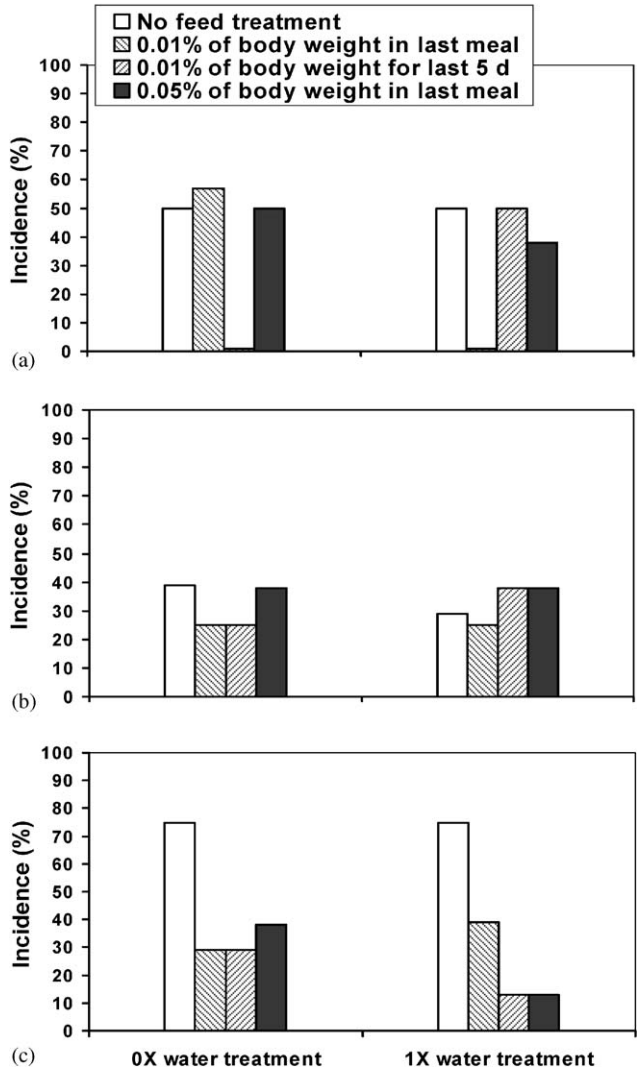

Fig. 3. Effects of administering sodium chlorate preparations in drinking water and/or feed on *Escherichia coli* O157:H7 prevalence in feces collected post treatment but immediately before shipment to slaughter (a) and in rumen contents (b) and feces collected at slaughter (c). Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1X water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests for main effects were done by logistic regression.

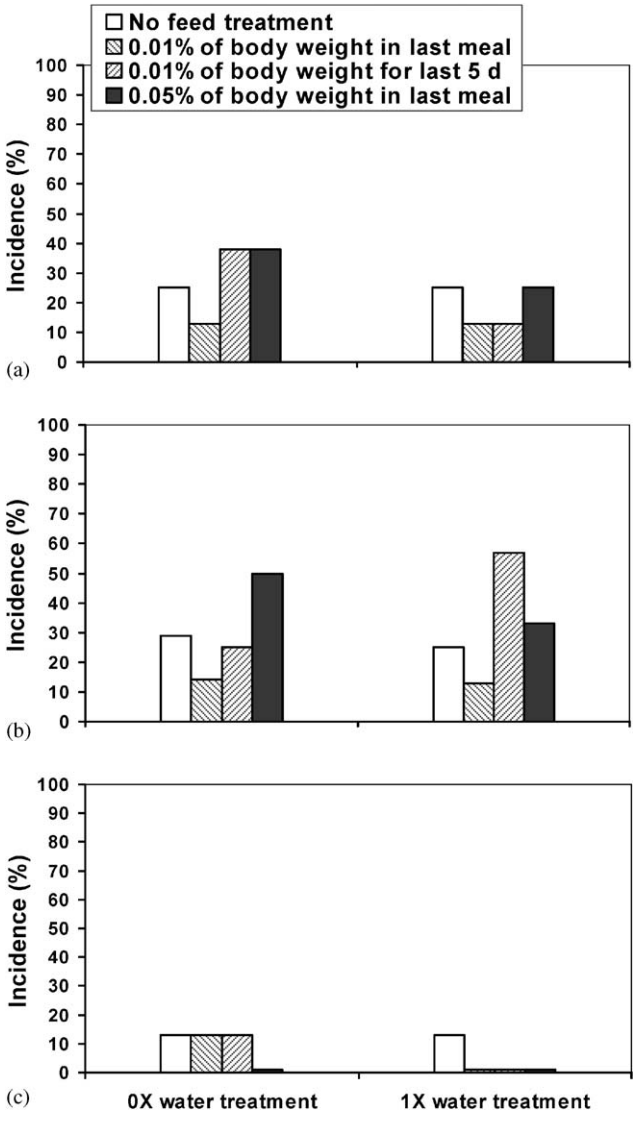

Fig. 4. Effects of administering sodium chlorate preparations in drinking water and/or feed on *Escherichia coli* O157:H7 prevalence in swabs collected from the back midline (a) and left rump area (b) of the hide and from the bung/hock area of the carcass (c) collected at slaughter. Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1X water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests for main effects were done by logistic regression.

($P = 0.015$) but not on the ratio of acetate to propionate ($P > 0.05$). Main effect means for acetate were 42.3 vs. 31.1, for propionate were 24.0 vs. 17.4 and for butyrate were 7.3 vs. 4.6 μmol/g content for animals administered 0 or 2500 ppm active chlorate ion, respectively (SEM = 2.7, 1.6 and 0.7 for acetate, propionate and butyrate, respectively). Main effects of feed treatment, replicate or any possible interactions were not observed on rumen volatile fatty acid concentrations ($P > 0.05$) and no effects ($P > 0.05$) were observed on fecal volatile fatty acid concentrations. LSD All-Pairwise Comparison means of rumen and fecal volatile fatty acid concentrations are presented in Tables 3 and 4.

4. Discussion

Results from the present study demonstrate that both water and feed administration of the experimental chlorate preparations was more effective in achieving total tract reductions in generic *E. coli* concentrations than administration of either alone. The fact that the water treatment resulted in reductions of ruminal but not fecal *E. coli* suggests that there was not enough time between initiation of water treatment and slaughter (< 18 h) for the chlorate consumed in the water to reach

Table 2
Effects of administering sodium chlorate preparations in drinking water and/or feed on feed and water intake[a]

Amount of experimental chlorate product in the feed	Daily feed intake during supplememntation period[b] (kg/d)		Water consumption (l/12 h period)	
	0X water treatment	1X water treatment	0X water treatment	1X water treatment
None	3.19[d]	4.25[c]	16.1[cd]	18.5[c]
0.01% Body weight in last meal	4.21[c]	3.48[cd]	18.9[c]	13.3[cd]
0.01% Body weight for last 5 d	3.88[cd]	3.78[cd]	10.5[d]	14.2[cd]
0.05% Body weight in last meal	3.94[cd]	4.42[c]	18.9[c]	16.1[cd]
SEM	0.35		2.62	

[a]Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1X chlorate water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests were done by analysis of variance and data presented are LSD All-Pairwise Comparisons of means.
[b]Dry matter basis.
[d]Columns with unlike superscripts differ at $P<0.05$.

Table 3
Effects of administering sodium chlorate preparations in drinking water and/or feed on concentrations of volatile fatty acids in rumen contents collected at slaughter[a]

Amount of experimental chlorate product supplemented to feed	Rumen concentrations (μmol/ml)							
	Acetate		Propionate		Butyrate		Acetate:Propionate	
	0X water treatment	1X water treatment	0X water treatment	1X water treatment	0X water treatment	1X water treatment	0X water treatment	1X water treatment
None	32.1[bc]	35.1[bc]	20.0[bcd]	18.6[cd]	6.4[bcd]	4.9[cd]	1.8	1.9
0.01% Body weight in last meal	45.9[b]	24.9[c]	24.7[bc]	14.8[d]	9.2[b]	3.0[d]	1.8	1.7
0.01% Body weight for last 5 d	45.7[b]	30.1[bc]	22.6[bcd]	17.5[cd]	5.9[bcd]	5.3[bcd]	2.0	1.8
0.05% Body weight in last meal	45.6[b]	34.1[bc]	28.7[b]	18.5[cd]	7.5[bc]	5.3[bcd]	1.7	1.7
SEM	5.94		3.35		1.54		0.19	

[a]Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1 X chlorate water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests were done by analysis of variance and data presented are LSD All-Pairwise Comparisons of means.
[cd]Columns with unlike superscripts differ at $P<0.05$.

Table 4
Effects of administering sodium chlorate preparations in drinking water and/or feed on concentrations of volatile fatty acids in feces collected at slaughter[a]

Amount of experimental chlorate product supplemented to feed	Fecal concentrations (μmol/ml)							
	Acetate		Propionate		Butyrate		Acetate:Propionate	
	0X water treatment	1X water treatment	0X water treatment	1X water treatment	0X water treatment	1X water treatment	0X water treatment	1X water treatment
None	41.0	44.4	14.3	17.4	3.3	3.1	2.8	2.6
0.01% Body weight in last meal	31.8	34.9	12.1	12.7	1.9	2.4	2.6	2.7
0.01% Body weight for last 5 d	35.3	29.5	11.4	13.3	2.9	2.7	2.9	2.2
0.05% Body weight in last meal	**38.4**	**29.1**	14.7	11.3	2.4	2.0	2.7	2.5
SEM	7.76		2.61		0.70		0.30	

[a]Feed treatments were provided as indicated and water treatments were provided 12 h immediately before shipment to slaughter. 0X and 1X chlorate water treatments contained 0 and 2500 ppm active chlorate ion, respectively. Tests were done by analysis of variance and data presented are LSD All-Pairwise Comparisons of means which did not differ ($P<0.05$).

the lower gastrointestinal tract. The fact that there was an effect of feed treatment on fecal but not ruminal *E. coli* concentrations suggests that some of the experimental chlorate product consumed 24–26 h earlier had bypassed the rumen as intended and reached the lower gastrointestinal tract by time of slaughter. Reductions of

generic *E. coli* concentrations in this study were less than those obtained in an earlier study (Callaway et al., 2002) most likely because of the lower amounts of chlorate administered in this study. Considering that meat products can be contaminated with *E. coli* originating from cattle feces of shedding animals (Aslam et al., 2003; Elder et al., 2000; Omisakin et al., 2003), it is reasonable to expect that reductions in *E. coli* colonization achieved here or elsewhere by the use of competitive exclusion bacteria (Brashears et al., 2003a, b; Zhoa et al., 1998, 2003), vaccination (Moxley et al., 2003) or antimicrobial interventions (Elder et al., 2003) would compliment multi-hurdle pathogen reduction strategies. In support of this rationale, an effect of feed treatment on reducing generic *E. coli* concentrations on the hide at the left rump area was observed in this study but whether or not this was a direct consequence of reduced fecal concentrations of *E. coli* can not be discerned from these data. Neither feed or water treatment affected generic *E. coli* concentrations on the back midline area of the hide but this was not unexpected considering the short duration of the treatment periods. While we did observe an effect of replicate on the recovery of *E. coli* from the hide and carcass, this most likely can be attributed to kill order, as cattle in the second and third replicates were killed later in the processing plant's daily kill order than the cattle in the first and fourth replicates. Our observation that water treatment reduced rumen *E. coli* concentrations and tended to reduce *E. coli* concentrations on the carcass suggests that *E. coli* originating from the rumen may pose a greater risk to carcass contamination than presently thought, despite observations here and elsewhere that the rumen harbors fewer *E. coli* than the lower gut (Grauke et al., 2002; Laven et al., 2003).

Our observation that feed treatment reduced the incidence of *E. coli* O157:H7 in feces collected at slaughter but not in feces collected 4–6 h earlier before the animals were shipped to slaughter suggests that a longer post-administration interval may be required to deliver chlorate to the terminal section of the bovine rectum which has been reported to preferentially harbor *E. coli* O157:H7 (Naylor et al., 2003). In support of this finding, generic *E. coli* concentrations in feces collected at the slaughter facility were generally reduced more than in feces collected 4–6 h earlier. While this again is likely due to the chlorate having more time to be present in the lower gut an effect of transportation per se cannot be ruled out. No other treatment effects against the recovery of *E. coli* O157:H7 were observed.

In the present study, we observed no adverse effects due to chlorate administration on feed intake or water consumption. However, feed intake was much lower than expected even for the animals receiving the control diet and this most likely occurred because the cattle had not adjusted sufficiently to our facilities or diet during the 1 week acclimation period. An effect of chlorate administration in drinking water was observed on ruminal but not fecal volatile fatty acid concentrations. The water treatment effect on rumen volatile fatty acid concentrations represents a stifling of a nonsignificant feed treatment effect as animals fed the experimental chlorate product but not administered chlorate in their water had markedly higher acid concentrations than those administered chlorate in their water.

Results from the present experiment demonstrate that the experimental chlorate preparations can be practically, easily and safely administered to feedlot cattle via feed and or water to reduce *E. coli* concentrations in the rumen, feces and on the hindquarters of the animal at slaughter. Moreover, at the concentrations used, these experimental preparations had no observable negative effect on animal performance or health, or on product quality (King et al., 2003). These findings are important since chlorate salts are toxic at high concentrations (Cosmetic Ingredient Review Panel, 1995; Radeleff, 1970). For instance, approximately 2–3 kg of crystalline sodium chlorate can kill a 400 kg animal. The experimental chlorate product used in this study had been formulated to possess a rumen bypass characteristic (i.e., a reduced rate of chlorate ion bioavailability) and thus a decreased toxicity. Likewise, the chlorate solution added to the drinking water was prepared so as to be safe for the animals. Still, regulatory issues need to be resolved, especially those pertaining to potential residues in product destined for human consumption, before these experimental chlorate preparations can be approved for commercial use.

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